Cryptic homoeology analysis in species and hybrids of genus *Zea*

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Abstract

Cryptic intergenomic pairing of genus *Zea* was induced by the use of a diluted colchicine solution, in order to elucidate the phylogenetic relations and differentiation of the homoeologous genomes. Results indicate that in species and hybrids with 2n = 20, there was chromosome pairing between the homoeologous A and B genomes with a maximum of 5IV, with the exception of *Zea diploperennis* and their interspecific hybrids, where cryptic homoeologous chromosome pairing was not induced. In almost all 2n = 30 hybrids, observed cryptic pairing increased to a maximum of 10III, although *Z. mays* x *Z. mays* with 2n = 30, did not show significant differences between treated and untreated materials. Pairing was also observed in species and hybrids with 2n = 40, in which a maximum of 10IV was observed, with

the exception of *Z. mays* with 2n = 40 where treated and untreated cells did not differed significantly.

Additional key words: colchicine, genome, maize, subgenome, teosinte.

Abbreviations: A – subgenome A *Zea;* B – subgenome B *Zea*; Bp1 – subgenome Bp1 *Zea perennis*; Bp2 – subgenome Bp2 *Zea perennis*; 2,4-D - 2,4 dichlorophenoxyacetic; I - chromosome monovalent; II - chromosome bivalent; III - chromosome trivalent; IV - chromosome quadrivalent; MDL - (*Zea mays x Zea diploperennis) x Zea luxurians;* MDP - *Zea mays* ssp *mays* 2n = 40 x (*Zea diploperennis x Zea perennis)* 2n = 40; *pam1* - gene *plural abnormalities of meiosis*; *Ph* – gene *pairing homoeologous; Ph1 – pairing homoeologous 1*; Zd - *Zea diploperennis;* ZdxZl - *Zea diploperennis x Zea luxurians;* ZdxZp30 - *Zea diploperennis x Zea perennis* 2n = 30; ZdxZp40 - *Zea diploperennis x Zea perennis* 2n = 40; Zl - *Zea luxurians;* ZlxZp30 - *Zea luxurians x Zea perennis* (2n = 30); Zm - *Zea mays* ssp *mays* 2n = 20; Zm40 - *Zea mays ssp mays* 2n = 40; Zmex - *Zea mays* ssp *mexicana*; ZmxZd - *Zea mays* ssp *mays* x *Zea diploperennis ;* ZmxZl - *Zea mays* ssp *mays* x *Zea luxurians;* ZmxZm30 - *Zea mays* ssp *mays* (2n = 40) x *Zea mays* ssp *mays* (2n = 20); ZmxZmex - *Zea mays* ssp *mays* x *Zea mays* ssp *mexicana;* ZmxZp30 - *Zea mays* ssp *mays* x *Zea perennis* 2n=30; ZmxZp40 - *Zea mays* 2n = 40 *x Zea perennis*; ZmxZpar - *Zea mays* ssp *mays* x *Zea mays* ssp *parviglumis;* Zp - *Zea perennis;* Zpar - *Zea mays* ssp *parviglumis;* ZpxZmex - *Zea perennis* x *Zea mexicana;* ZparxZd40 - *Zea mays* ssp *parviglumis x Zea diploperennis* 2n = 40.

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Introduction

Zea is an important genus of the tribe Maydeae. According to Doebley and Iltis (1980), Iltis and Doebley (1980) and Iltis and Benz (2000) it is composed of two section: Section Luxuriante, which includes the annuals teosintes *Zea luxurians* and *Zea nicaraguensis* and the perennials *Zea diploperennis* and *Zea perennis* and Section Zea comprises only an annual species (*Z. mays* L) which can be divided into three subspecies: *Z. mays* ssp*. mays* (maize) and the teosintes *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis.* All the species mentioned above have 2n = 20 chromosome except Z*. perennis* which has 2n = 40.

The maize genome is characterized by having a large number of duplicated genes (Wendel 2000). Three models can explain the large-scale duplications in the maize genome, that is, segmental duplication (multiple independent duplications within a genome), autotetraploidy (intraspecific genomic duplication), and allotetraploidy (interspecific genome hybridization).

Swigonová *et al*. 2004, support a theory in which maize has a tetraploid origin. This analysis also indicates a contemporaneous divergence of the ancestral sorghum genome and the two maize progenitor genomes about 11.9 million years ago (Mya). On the basis of a putative conversion event detected for one of the genes, tetraploidization must have occurred before 4.8 Mya, and therefore, the major maize genome expansion.

According to Schnable *et al.* 2011 and Schnable and Freeling 2011, maize is a tetraploid species with two differentiated parental genomes, maize1 and maize2. Maize1 is the subgenome that experienced less gene losses followed by the whole genome duplication in maize lineage 5-12 Mya. Genes located on this subgenome tend to be expressed at a higher level in modern maize (Schnable and Freeling 2011).

Colchicine concentrations of 0.5 x 10^{-4} M induce homoeologous chromosome pairing (Poggio *et al.* 1990, Molina 2011), with the formation of heteromorphic bivalents or multivalents (Driscoll *et al* 1967, Feldman and Avivi 1988), which favors intergenomic pairing in species with homoeologous genomes (Dover and Riley 1973).

Driscoll and Darvey (1970) observed that colchicine affects the spatial relationship of homoeologous chromosomes but not the formation of chiasmata. Additionally, they suggested that chromosome position is crucial in the meiotic pairing of homologous and homoeologous chromosomes, altering the arrangement of chromosomes in the nuclear membrane, and allowing the expression of cryptic genomic homology.

Jackson (1982) described a model that explains the chromosome pairing and chiasma formation in homologous and homoeologous genomes, in which there is a genetic control of the specific binding site of chromosomes to the nuclear membrane, under the regulation of the *Ph* gene. Jackson and Murray (1983) demonstrated that the application of diluted colchicine solution to meiotic cells can break the genetic control of these genes, promoting the intergenomic pairing, and thus revealing the cryptic homology in polyploids.

In maize, the *pam1* gene (*plural abnormalities of meiosis 1*) is associated with the formation of the bouquet by intervening in the telomere anchoring to the nuclear membrane, and by facilitating homologous chromosome pairing. Golubovskaya *et al.* (2002) concluded that the *pam1* gene plays an important role in the formation of the bouquet and in the pairing of homologous chromosomes.

Several researchers have treated premeiotic cells of *Zea mays, Z. perennis*, *Z. diploperennis* and their hybrids with diluted colchicine solutions (Poggio *et al.* 1990, Molina and García 1999, 2000, 2001, Molina *et al.* 2004, 2005, Molina 2011, González and Poggio 2011). Up to 5 quadrivalents have been observed in maize and hybrids with 2n = 20, while a higher number

of quadrivalents has been found in *Zea perennis* when compared to untreated controls. Colchicine treatment has been observed to favor homoeologous chromosome pairing, and more than one mechanism has been proposed to explain such effect, modification of the position of the chromosomes in the nuclear membrane or alteration in the formation of the bouquet (Bass *et al.* 2000), and annulment of the expression of a maize gene, equivalent to the *Ph* gene of wheat (Poggio *et al.* 1990, Molina *et al.* 2004).

In the present study, cryptic homology in species and hybrids of the genus *Zea* was analyzed, with the aim to explore the phylogenetic relationship among Zea species, by using diluted colchicine solutions that induce intergenomic chromosome pairing.

MATERIALS AND METHODS

MATERIALS

PARENTAL SPECIES

Section Zea

***Zea mays* ssp *mays* 2n = 20 (Zm):** Open pollinated population Colorado Klein. Inbred line knobless, from the Maize Genetic Cooperation Stock Center (MGCSC), Urbana, Illinois, USA.

***Zea mays ssp mays* 2n = 40 (Zm40)**. Sugary inbred lines N103A, N104B, N107C, N107B, and 90-2189-2190, from MGCSC.

***Zea mays* ssp *mexicana* 2n = 20 (Zmex)**. From CIMMYT (Mexico).

***Zea mays* ssp *parviglumis* 2n = 20 (Zpar)**. From Dr. Bird, CIMMYT (Mexico).

Section Luxuriantes

***Zea luxurians* 2n = 20 (Zl).** From Guadalajara, Mexico.

***Zea diploperennis* 2n = 20 (Zd)**. From Sierras Occidentales de Manjatlan, Jalisco, Mexico. Courtesy of Dr. Iltis.

***Zea perennis* 2n = 40 (Zp)**. From Ciudad Guzmán, Jalisco, Mexico. Courtesy of Dr. Prywed, introduced in 1962 at the Instituto Fitotécnico de Santa Catalina, Llavallol, Argentina.

HYBRIDS

Dihybrids

2n = 20

Zea mays ssp *mays* x *Zea mays* ssp *mexicana* (ZmxZmex).

Zea mays ssp *mays* x *Zea mays* ssp *parviglumis* (ZmxZpar).

Zea mays ssp *mays* x *Zea luxurians* (ZmxZl).

Zea mays ssp *mays* x *Zea diploperennis* (ZmxZd).

Zea diploperennis x Zea luxurians (ZdxZl).

2n = 30

Zea mays ssp *mays* (2n = 40) x *Zea mays* ssp *mays* (2n = 20), (ZmxZm30).

Zea mays ssp *mays* (2n = 40) x *Zea parvig*lumis (ZmxZpar30).

Zea mays ssp *mays* x *Zea perennis* (ZmxZp30).

Zea diploperennis x Zea perennis (ZdxZp30).

Zea luxurians x Zea perennis (ZlxZp30).

Zea perennis x Zea mexicana (ZpxZmex).

2n = 40

Zea mays with 2n = 40 *x Zea perennis* (ZmxZp40).

Zea mays ssp *parviglumis x Zea diploperennis* 2n = 40, obtained by chromosome duplication of the 2n = 20 hybrid (ZparxZd40).

Zea diploperennis x Zea perennis, obtained by crossing a non-reduced gamete of *Zea diploperennis* with a normal gamete of *Zea perennis* (ZdxZp40).

Trihybrids

2n = 20

A cross between hybrid (*Zea mays x Zea diploperennis) x Zea luxurians* (MDL).

2n = 40

Zea mays ssp *mays* 2n = 40 x (*Zea diploperennis x Zea perennis)* 2n = 40 (MDP).

METHODS

Field experiments

Crosses and self pollinations of maize inbred lines and *Teosinte* populations were made at field and greenhouse conditions. When *Teosinte* was used as male, before pollination, maize silks were cut to 3 or 4 cm, since the maximum length of the pollinic tube in *Teosinte* is about 6 to 7 cm, unlike maize, whose pollinic tube can reach more than 30 cm. In crosses between species whose hybrids have a chromosome number of 2n = 30, which are generally difficult to obtain due to a limited embryo growth, a solution of 0.45 mM of 2,4 dichlorophenoxyacetic (2,4-D) (Furini and Jewel 1995) was applied to the ears, two days after pollination, in order to favor embryo development.

Rescue and embryo culture

Immature embryos from 2n = 30 hybrids were rescued and cultured *in vitro*. About 12 to 30 days after fecundation, the embryo collapsed and died. The survival period depended on the environmental conditions. If the plants are grown under optimal field conditions for maize development, embryos can survive for 12 days, whereas under greenhouse conditions during winter, the development of embryos is generally delayed and survival is longer (21 to 30 days).

Ears were harvested when embryos had a maximum length of 1 mm**.** Caryopses were washed with a 2.5% sodium hypochlorite solution and then sown in a basic medium (García and Molina 1992, 2001) supplemented with 4.5 µmol dm^{-3} de 2,4-D. Treated caryopses were incubated in a growth chamber with a photoperiod of 16 h light and 8 h of darkness, subcultured every 30 days in a callus maintenance medium (García and Molina 1992).

Regenerated plants were transplanted into a culture medium free of 2,4-D to develop roots. Subsequently, plants were transplanted and acclimated in a greenhouse.

Treatment with diluted colchicine solution

Immature tassels from different *Zea* species and hybrids were cut and introduced in a 0.5 x 10^{-4} M colchicine solution for 12 h, and then stored in distilled water for 24 h (controls were stored for 36 h in distilled water). Then, the treated material and the controls were fixed in Farmer solution (3:1 ethylic alcohol/acetic acid), where they were conserved for 8 to 10 days, and then stored at 4 °C in an alcohol 70 solution.

Cytogenetic analysis

To analyze the meiotic configurations in parental species and hybrids, anthers excised from male florets previously fixed in Farmer solution were squashed in a drop of 2 % ferric hematoxylin, using a micro-drop of ferric acid as mordant. Between 130 and 224 cells were analyzed in controls and treatments.

Significant differences between meiotic configurations (control vs. the corresponding treatment) were tested using the Mann-Whitney U test (Sokal and Rohlf 1978) at a 5% probability level (STATISTICA program version 7).

Chromosomal notation

I (monovalent); II (bivalent); III (trivalent); IV (quadrivalent)

Results

Effect of the colchicine diluted solution on the cryptic pairing of the Zea complex

To obtain the cryptic pairing of homoeologous chromosomes of different genomes from species and hybrids of the *Zea* complex, tassels were treated with a diluted colchicine solution, with the following results:

In Zea species with 2n = 20:

In *Z. mays* with 2n = 20 (Table 1A), *Z. mexicana* (Table 1B), *Z. parviglumis* (Table 1C) and *Z. luxurians* (Table 1D), colchicine induced the homoeologous chromosome pairing of the two relict genomes, designated as genomes A and B (Fig. 2), with a maximum of 5IV (Table 1A, 1B, 1C, 1D and Fig. 1A). The exception was *Z. diploperennis* (Table 1E), in which no quadrivalents formation was observed using the colchicine concentration that showed quadrivalents in the other species.

In Zea hybrids with 2n = 20:

Cryptic pairing was observed in *Z. mays x Z. mexicana* (Table 2A), *Z. mays x Z. parviglumis* with 2n = 20 (Table 2B), *Z. mays x Z. luxurians* (Table 2C) and the trihybrid (*Z. mays x Z. diploperennis) x Z. luxurians* (Table 2F), although in different proportions depending on the hybrid analyzed. The highest percentage of pairing was observed in *Z. mays* x *Z. parviglumis* with 2n = 20 (Table 2B). On the other hand, cryptic pairing of homoeologous chromosomes of the A and B genomes was not observed in *Z. mays x Z. diploperennis* with 2n = 20 (Table 2D) and *Z. diploperennis* x *Z. luxurians* hybrids (Table 2E). In both cases, one of the parents was *Z. diploperennis,* which also showed no induction of homoeologous chromosome pairing (Table 1E).

In Zea hybrids with 2n = 30:

No significant differences between treated material and controls were observed in the hybrid between *Zea mays* with different ploidy levels (Table 3A). The most frequent configuration was 8III+2II+2I. In the hybrids *Z. mays x Z. parviglumis* with 2n = 30 (Table 3B) and *Z. luxurians x Z. perennis* with 2n = 30 (Table 3E), cryptic pairing was increased, with a higher percentage of trivalents in the treated material, in comparison with the controls. A increase in cryptic pairing was also observed in *Z. perennis x Z. mexicana* (Table 3D) and *Z. diploperennis x Z. perennis*

with 2n = 30 (Table 3F). While treated material showed up to 10III, a maximum of 6III were observed in the controls (Table 3F). The highest difference between treated genotypes and controls was observed in the 2n = 30 hybrid *Z. mays x Z. perennis* (Table 3C), where the meiotic configuration most frequently observed was 5III+5II+5I for controls and 8III+2II+2I for the treated hybrids.

In species and hybrids with 2n = 40**:**

In the 2n = 40 maize, only a slight increase in the meiotic configuration of 9IV+2II or 10IV, and a minimal decrease in the configurations with less than 7IV was observed (Table 4A). The most frequent meiotic configuration in *Z. perennis* controls was 5IV+10II, and less frequently 6IV+8II. In the treated material, the percentage of quadrivalents increased to a maximum of 10, with an average of 7IV+6II (Table 4B).

In *Z. mays x Z. perennis* hybrids with 2n = 40 (Table 4C and Fig**.** 1D), *Z. diploperennis* x *Z. perennis* with 2n = 40 (Table 4D) and the trihybrid *Z. mays x* (*Z. diploperennis x Z. perennis*) (Table 4F), there was a increase in the number of IV treated material, reaching a maximum of 10IV. In all cases, controls showed a very low proportion of pairing among homoeologous chromosomes, especially between *Z. diploperennis* and *Z. perennis* (Table 4D).

The behavior of the hybrid *Z. parviglumis x Z. diploperennis* with 2n = 40, obtained by chromosome duplication of *Z. parviglumis x Z. diploperennis* with 2n = 20, was different from that of the rest of the hybrids with the same chromosome number (Table 4E). The most frequent meiotic configuration in the control was 2IV+16II. Apparently, pairing of identical chromosomes in this hybrid was favored, reducing the homoeologous pairing between the A and B genomes, which could explain the low frequency of IV observed. The treated material showed results similar to the rest of the hybrids studied (Table 4E).

In all the cases, the number of chiasma increased in the treated material (Table 5), except in *Z. diploperennis,* maize with 2n = 40, the hybrid *Z. mays* x *Z. mays* 2n = 30, *Z. mays x Z. diploperennis*, and *Z. diploperennis x Z. luxurians* with 2n = 20 (Table 5).

Discussion

Cryptic pairing among homoeologous chromosomes of the genus *Zea*

The analysis of the meiotic behavior of *Zea* species with 2n = 20 after treatment with a diluted colchicine solution (Table 1A, 1B, 1C and 1D) showed homoeologous chromosome pairing in all species (with a maximum of 5IV), with the exception of *Zea diploperennis,* which did not show significant differences when compared to the treated and controls (Table 1E).

More than one possible reason can be mentioned to explain the lack of pairing in *Zea diploperennis.* First, the expected alteration of the spatial relation of chromosomes (Driscoll and Darvey 1970) was not observed, hence, they were able to maintain their ordering (Feldman and Avivi 1988, Feldman *et al.* 1997) and anchor to the nuclear membrane (Bass *et al.* 2000, Chikashige *et al.* 2010), favoring pairing among homologous chromosomes. Second, the *pam1* gene, which intervenes in the presynaptic mechanisms (Felman and Avivi 1988, Bozza and Pawlowski 2008) and in the chromosome reorganization related to the formation of the bouquet (Zickler and Kleckner 1998, Bass *et al.* 2000), may have a different effect on *Z. diploperennis* than on the other *Zea* species. Third, the action of an equivalent *Ph* gene in *Z. diploperennis* is not inhibited by the diluted colchicine solution used. Fourth, *Z. diploperennis* chromosomes are not homoeologous, which is possible if *Z. diploperennis* was obtained from a haploid *Z. perennis.* Independently of which is the mechanism that resulted in the lack of pairing observed, it would be interesting to analyze the last hypothesis in future researches.

Cryptic pairing of homoeologous chromosomes in 2n = 20 hybrids of the genus *Zea* (Table 2) varied according to the cross analyzed, but was higher in the hybrids between more closely

related species (*Z. mays, Z. mexicana* and *Z. parviglumis*) than in more distant species (*Z. luxurians* and *Z. diploperennis*).

The hybrids *Z. mays x Z. luxurians* (Table 2C) and (*Z. mays x Z. diploperennis*) *x Z. luxurians* (Table 2F) showed a similar cryptic pairing, but resulted lower than in species with higher chromosome affinity. The cryptic pairing observed in the hybrid *Z. mays* x *Z. luxurians* (Table 2C) maybe attributable to a higher differentiation among their homoeologous chromosomes, since parental species are evolutionarily more distant.

The meiotic behavior of *Zea* in the species and hybrids with 2n = 30 after treatment with colchicine showed no significant differences between treated and untreated material in the hybrids between species of *Z. mays* (Table 3A). A possible explanation is the fact that this hybrid is a cross between genotypes of the same species with different ploidy level, and the preferential pairing is between homologous chromosomes. The preferential pairing of homologous chromosomes was observed by Santos and Orellana 1983, Santos *et al.* 1984, Jenkinsy and Chatterjee 1994, Jenczewski and Alix 2004. However, it is not clear why 2n = 20 maize showed cryptic pairing between the A and B genomes (Table 1A and Fig. 1A), which was inhibited with a higher ploidy level (Table 4A).

A high level of homoeologous chromosome pairing, with a maximum of 10III, was detected in untreated *Z. mays* x *Z. parviglumis* with 2n = 30 (Table 3B) and *Z. luxurians* x *Z. perennis* (Table 3E). Colchicine increased the percentage of III only slightly.

The highest response to colchicine treatment, with a maximum of 10III, was obtained in the 2n = 30 hybrid *Z. mays* x *Z. perennis* (Table 3C and Fig. 1C), suggesting that chromosomes of *Z. mays* and *Z. perennis* are homoeologous, even though both species are evolutionarily differentiated.

Different results were found in 2n = 40 species and hybrids. Treated and untreated *Z. mays* showed pairing between A and B homologous chromosomes, with a maximum of 10IV (Table 4A). Colchicine treatment did not induce homoeologous pairing and no hexavalents or octovalents chromosomes were observed (Table 4A).

Treatment in *Z. perennis* induced only pairing of homoeologous chromosomes of the B genome (Table 4B and Fig. 1B), with a maximum of 10IV. Genome B chromosomes paired as bivalents, while genome A chromosomes paired as quadrivalents. There was no pairing between chromosomes of the A and B genome.

Considering *Zea mays* with 2n = 40 (Table 4A), *Z. diploperennis* x *Z. perennis* 2n = 40 (Table 4D), *Z. mays* x *Z. perennis* 2n = 40 (Table 4C and Fig.1D) and *Z, mays x (Z. diploperennis x Z. perennis)* (Table 4F), chromosomes of the A genome from species that form the hybrids paired similarly in both treated and untreated material. Colchicine induced the cryptic pairing of homologous chromosomes of the B genome, with a maximum of 10IV.

Possible evolution of the genomes of Zea

Previous results (Molina and Naranjo 1987, Molina and García 2001, Molina *et al.* 2004, Molina 2011, Poggio *et al.* 1990, Naranjo *et al.* 1989, 2004, González and Poggio 2011) support the theory that consider *Zea* as an autotetraploid with two subgenome with different level of conservation (Swigonová *et al*. 2004, Schnable and Freeling 2011, Schnable *et al*. 2011).

A possible mechanism of differentiation of subgenomes which could have led to different *Zea* species would consist in a duplication of the A genome of a diploid species, followed by a differentiation in the homoeologous genomes A and B (Fig. 2). Another possible mechanism that resulted in the genotypic constitution of AA BB individuals is an ancestral cross between two diploid species (AA and BB). Our results support the theory of a chromosome duplication

followed by a differentiation of two genomes, since in the hybrid *Zea mays* ssp. *parviglumis* x *Zea diploperennis* with 2n = 40 (obtained by crossing two Zea species and then duplicated) chromosomes of each species paired preferentially with the homologous instead of homoeologous chromosomes, resulting in a more frequent configuration of 2IV+16II, while in most hybrids the configuration was 5IV+10II. The A genome would have remained undifferentiated, allowing the current pairing of homoeologous chromosomes of interspecific hybrids as if they were homologous. The differentiation of B genomes would be the evolutionary process ((Fig. 2) which would have resulted in different species of *Zea*, probably favoring their geographic isolation (Ruiz *et al.* 2001, Fukunaga *et al*. 2005) or domestication (Swanson-Wagner *et al.* 2012)

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Species	IV/Cell						Nº of cells studied
	0	1	2	3	4	5	
A - *Zea mays*							
Control	100.0	0.0	0.0	0.0	0.0	0.0	200
Treated	38.3	17.8	15.3	8.2	16.8	3.6	196
B - *Zea mexicana*							
Control	100.0	0.0	0.0	0.0	0.0	0.0	136
Treated	37.7	25.7	18.0	7.8	6.6	4.2	167
C - *Zea parviglumis*							
Control	100.0	0.0	0.0	0.0	0.0	0.0	181
Treated	34.3	20.0	17.8	12.0	13.1	2.8	175
D - *Zea luxurians*							
Control	100.0	0.0	0.0	0.0	0.0	0.0	162
Treated	36.5	23.0	19.6	10.1	6.1	4.7	148
E - *Zea diploperennis*							
Control	100.0	0.0	0.0	0.0	0.0	0.0	164
Treated	100.0	0.0	0.0	0.0	0.0	0.0	196

Table 1: Percentage of quadrivalents in parental species with 2n = 20, for plants treated with colchicine (0.5 x 10^{-4} M) and untreated control plants (significance in parenthesis) : *A- Zea mays* (p-value 0.00); *B- Zea mexicana* (p-value 0.00); *C- Zea parviglumis* (p-value 0.00); *D- Zea luxurians* (p-value 0.00); *E- Zea diploperennis* (p-value 1.00).

Hybrids	IV/Cell						Nº of cells studied
	0	1	2	3	4	5	

A - ZmxZmex							
Control	100.0	0.0	0.0	0.0	0.0	0.0	127
Treated	35.2	18.6	22.8	3.4	9.7	10.3	145
B - ZmxZpar							
Control	100.0	0.0	0.0	0.0	0.0	0.0	177
Treated	31.1	36.7	9.4	8.3	8.9	5.6	180
C - ZmxZl							
Control	100.0	0.0	0.0	0.0	0.0	0.0	200
Treated	59.0	15.4	13.4	4.4	4.9	2.9	203
D - ZmxZd							
Control	100.0	0.0	0.0	0.0	0.0	0.0	181
Treated	100.0	0.0	0.0	0.0	0.0	0.0	179
E - ZdxZl							
Control	100.0	0.0	0.0	0.0	0.0	0.0	154
Treated	100.0	0.0	0.0	0.0	0.0	0.0	157
F - MDL							
Control	100.0	0.0	0.0	0.0	0.0	0.0	166
Treated	62.0	8.8	11.1	9.9	5.3	2.9	171

Table 2: Percentage of quadrivalents in hybrids with 2n = 20, for plants treated with colchicine (0.5 x 10^{-4} M) and untreated control plants (significance in parenthesis): *A- Zea mays* x *Zea mexicana* (p-value 0.00)*; B- Zea mays* x *Zea parviglumis* with 2n = 20 (p-value 0.00); *C- Zea mays* x *Zea luxurians* (p-value 1.25 x 10^{-13})*; D- Zea mays* x *Zea diploperennis* with 2n = 20 (p-value 1.00); *E- Zea diploperennis* x *Zea luxurians* (p-value 1.00); *F-* Trihybrid between (*Zea mays x Zea diploperennis*) x *Zea luxurians* (p-value 1.60 x 10^{-11})*.*

Hybrids	III/Cell											Nº of cells Studied
	0	1	2	3	4	5	6	7	8	9	10	
A - ZmxZm30												
Control	0.0	2.8	2.2	2.2	2.8	8.8	7.7	12.1	38.1	17.2	6.1	181
Treated	0.0	2.1	2.1	4.2	5.2	6.8	6.8	7.8	41.1	17.2	6.7	192
B - ZmxZpar30												

Control	5.1	7.2	11.6	13.8	19.6	29.0	5.7	3.6	2.2	0.0	2.2	138
Treated	0.0	1.9	7.1	7.7	14.7	32.1	12.8	11.5	6.4	3.8	2.0	156
C - ZmxZp30												
Control	0.0	0.0	0.0	2.8	30.0	59.3	5.7	2.2	0.0	0.0	0.0	140
Treated	0.0	0.0	0.0	0.0	4.7	5.3	12.4	20.6	29.4	15.3	12.3	170
D - ZpxZmex												
Control	0.0	0.0	3.8	5.0	26.1	58.4	6.7	0.0	0.0	0.0	0.0	180
Treated	0.0	0.0	0.0	2.9	13.8	56.3	6.9	6.3	5.2	5.2	3.4	174
E – ZlxZp30												
Control	3.1	4.4	8.1	6.9	23.1	26.2	11.9	8.1	5.0	1.9	1.3	160
Treated	0.0	0.0	3.9	5.1	10.0	22.2	21.1	18.8	11.1	5.0	2.8	180
F - ZdxZp30												
Control	0.0	0.0	4.2	4.8	18.1	69.9	3.0	0.0	0.0	0.0	0.0	166
Treated	0.0	0.0	0.0	0.0	9.1	44.0	21.2	8.0	5.7	6.9	5.1	175

Table 3: Percentage of trivalents in parental species with 2n = 30, for plants treated colchicine (0.5 x 10^{-4} M) and untreated control plants (significance in parenthesis): *A*- Hybrid between maize species with different ploidy level (p-value 0.77); *B- Zea mays* x *Zea parviglumis* with 2n = 30 (p-value 1.13 x 10^{-8}); *C- Zea mays* x *Zea perennis* with 2n = 30 (p-value 0.00); *D- Zea perennis* x *Zea Mexicana* (p-value 1.53 x 10^{-8})*; E- Zea luxurians* x *Zea perennis* (p-value 1.42 x 10^{-10})*; F- Zea diploperennis* x *Zea perennis* with 2n = 30 (p-value 0.40 x 10^{-16}).

IV/Cell											Nº of cells studied	Species and Hybrids
0	1		2	3	4	5	6	7	8	9	10		
		A – Zm40										
0.0	0.0	Control	0.0	2.1	0.7	4.2	6.3	10.6	16.9	26.1	33.1	142

0.0	0.0	Treated	0.0	0.0	2.2	3.9	5.1	7.9	16.8	27.0	37.1	178
		B - Zp										
0.0	2.3	Control	2.8	6.7	28.1	52.8	7.3	0.0	0.0	0.0	0.0	178
0.0	0.0	Treated	2.0	3.9	10.7	19.5	31.7	18.5	9.9	1.9	1.9	205
		C - ZmxZp40										
3.1	5.8	Control	9.4	20.6	21.4	34.8	4.9	0.0	0.0	0.0	0.0	224
0.0	0.0	Treated	5.0	21.0	26.0	32.0	5.0	4.0	4.0	2.0	1.0	200
		D - ZdxZp40										
1.9	2.8	Control	6.1	19.2	26.6	31.8	9.8	1.8	0.0	0.0	0.0	214
0.0	0.0	Treated	2.0	4.9	5.9	27.9	26.3	15.3	10.8	3.9	3.0	204
		E – ZparxZd40										
15.1	26.9	Control	29	17.7	11.3	0.0	0.0	0.0	0.0	0.0	0.0	186
0.0	7.8	Treated	16.7	18.9	15.6	11.1	13.9	5.0	3.3	4.4	3.3	180
		F - MDP										
1.2	8.0	Control	6.2	11.1	11.7	46.9	13.0	1.9	0.0	0.0	0.0	162
0.0	0.0	Treated	1.9	5.0	6.9	30.0	28.1	11.2	8.1	5.0	3.8	160

Table 4: Percentage of quadrivalents in parental species and *Zea* hybrids with 2n = 40, for plants treated with colchicine (0.5 x 10^{-4} M) and untreated control plants (significance in parenthesis): *A- Zea mays* with 2n = 40 (p-value 0.33); *B- Zea perennis* (p-value 0.00); *C- Zea mays x Zea perennis* with 2n = 40 (p-value 2.50 x 10^{-5}); *D- Zea diploperennis* x *Zea perennis* with 2n = 40 (p-value 0.00); *E- Zea parviglumis x Zea diploperennis* with 2n = 40 (p-value 0.00); *F-* trihybrid between *Zea mays* x (*Zea diploperennis x Zea perennis*) with 2n = 40 (p-value 1.10 x 10^{-16}).

Species and Hybrids	2n	Chiasmas	
		Control	Treated
Zm	20	14.00	20.00
Zmex	20	16.48	21.70

Zpar	20	15.36	20.08
Zl	20	15.34	21.34
Zd	20	14.00	14.64
Zm x Zmex	20	16.75	22.72
Zm x Zpar20	20	15.42	21.06
Zm x Zl	20	15.48	20.36
Zm x Zd20	20	15.00	15.35
Zd x Zl	20	15.26	16.24
MDL	20	14.57	19.38
Zm x Zm30	30	28.12	29.03
Zm x Zpar30	30	25.60	36.70
Zm x Zp30	30	23.06	37.09
Zp x Zmex	30	24.02	32.50
Zl x Zp	30	21.36	29.60
Zd x Zp30	30	17.25	26.34
Zm40	40	33.75	34.25
Zp	40	34.56	41.80
Zm x Zp40	40	33.59	42.50
Zd x Zp40	40	31.42	40.36
Zpar x Zd40	40	28.20	37.25
MDP	40	33.81	42.25

Table 5: Average number of chiasmata in species and hybrids of *Zea* in material treated with a diluted colchicine solution and untreated control

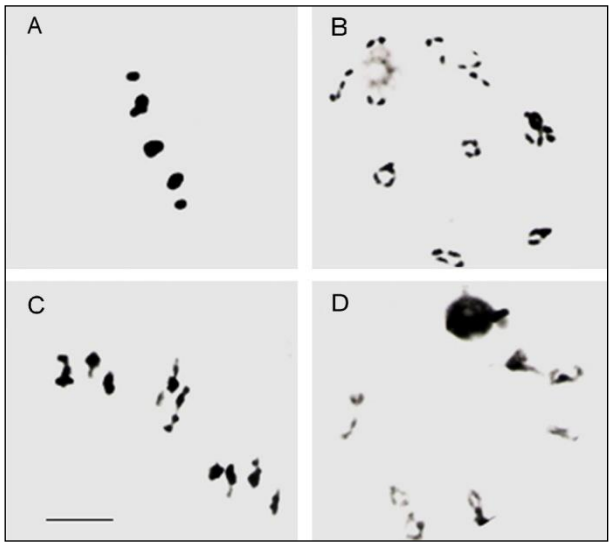

Fig. 1. Meiotic configurations induced by colchicine treatment in*: A- Z. mays* with 2n = 20 (5IV); *B- Zea perennis* (81V + 4II); *C- Z. mays* x *Zea perennis* with 2n = 30 (10III); *D- Z. mays* x *Zea perennis* with 2n = 40 (9IV + 2II). Scale 10 µm.

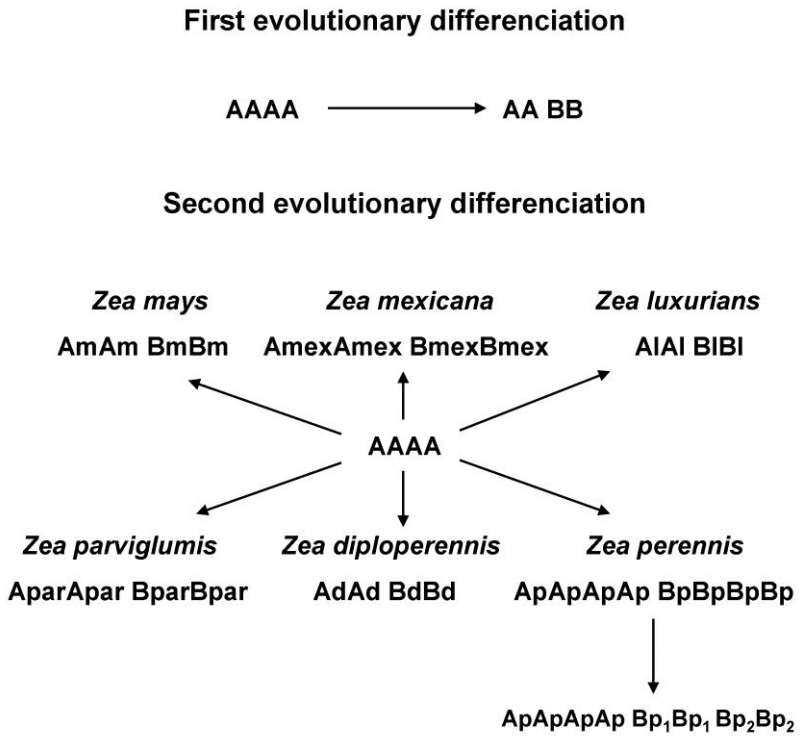

Fig. 2: Possible mechanisms of chromosome differentiation in the genus *Zea*. **First differentiation**: Tetraploid species with A genome would have differentiated into A and B genomes. **Second differentiation**: B genomes would have mutated or differentiated between them, resulting in different *Zea* species with 2n = 20. Particularly in the case of *Zea perennis*, chromosomes of the B genome could have undergone further differentiation, which resulted in Bp_1 and Bp_2 homoeologous chromosomes.